COMPETENT CELLS (H.M.M.: based on Hanahan method)

→ STREAK a fresh plate the day before inoculation from parent stock (NOT previous batch of competent cells: glycerol stock only) with appropriate antibiotic using sterile technique and grow O/N 37^0C.

--XL1 or 2 Blue= TetR
--TOP-10=StrepR (I plate without antibiotic for TOP-10)
--Other strains: check with parent company (website). Most of our strains in the stock box are from Invitrogen or Stratagene

→ INOCULATE a single colony into 5 ml **TYM** + antibiotic in a 14 ml Falcon 2059 tube; grow in shaker incubator at 37^0C, 225 RPM.
→ INOCULATE (in a.m.) 1 ml O/N culture into 500 ml **TYM** prewarmed to 37^0C in a 2 l Erlenmeyer flask (NO antibiotic!!); grow in shaker incubator at 37^0C, 225 RPM.
→ GROW until OD550= 0.5 (approximately 2.5 hours); take sample to measure OD but keep the culture shaking and at 37^0C
→ COOL culture rapidly in ice water, 2' with swirling
→ TRANSFER culture to pre-chilled 50 ml conical tubes
→ SPIN 3K, 10', 4^0C: NO BRAKE! (swinging bucket rotor –pre cool)
→ REMOVE SUP, CAREFULLY tap pellet and GENTLY resuspend in 20 ml **Tfb1**/tube

- try to get rid of clumps, but always be gentle

→ INCUBATE ON ICE in cold room for 1.5 hours
→ SPIN 3K, 10', 4^0C: NO BRAKE!
→ REMOVE SUP, CAREFULLY tap pellet and GENTLY resuspend in 1.5 ml **Tfb2**/tube

- complete resuspension critical, be gentle

→ ALIQUOT 200 μl to pre-labeled and pre-chilled eppendorfs
→ FREEZE ON DRY ICE and STORE immediately at -80^0C
→ Calculate cfu/μg DNA using 3 serial 1/10 dilutions of 1 ng/μl pUC18

TYM: autoclave

2% Bacto-Tryptone
0.5% Bacto-Yeast
0.1 M NaCl
0.01 M $MgCl_2$

Tfb1: filter sterilize, store at 4^0C
Make from autoclaved stocks
30 mM KOAc
50 mM $MnCl_2 4H_20$
100 mM KCl
10 mM $CaCl_2$
15% ***w/v*** glycerol
mix well, pH to 5.8 w/0.2 M acetic acid

Tfb2: filter sterilize, store at 4^0C

Make from autoclaved stocks

10 mM Na-MOPS pH7.0

10 mM KCl

75 mM $CaCl_2$

15% ***w/v*** glycerol